REMARKS

Reconsideration and reexamination of the subject application are respectfully requested in light of the foregoing amendments and following remarks. Amendments to the claims are made without disclaimer or prejudice to Applicants' rights to protect canceled subject matter in a later-filed continuing application.

1. Status of the Claims

Claims 1-17 are currently pending and stand rejected. By entry of the present amendment, claims 6, 7, 14, and 15 are canceled.

2. Support for the Amendments

The amendments to the claims are supported throughout the specification as filed and do not enter new matter into the application. The amendments to claim 1 subsume step (1) under step (2) and replace "removing said staining reagent" with "destaining said staining reagent." The term "destaining" is defined at page 11, lines 17-23, of the specification. A similar amendment is made to claim 10.

The amendment of claims 6 and 14 (staining reagent not covalently bound to the protein-binding membrane) is supported throughout the specification. *See, e.g.*, Specification, page 7, lines 20-21 ("The method employs a dye reagent that is rapidly removed from most protein-binding membranes. . . ."); page 11, lines 17-23 and page 14, line 18 *et seq.*(destaining); page 15, line 30, *et seq.* (reversing staining); page 17, line 13, *et seq.* (compound of formula I supplied separately from the protein binding membrane); page 19, lines 18-22 (example).

3. Restriction/Election

Applicants appreciate the Examiner's withdrawal of the requirement for restriction/election mailed March 29, 2007, following Applicants' traverse filed May 2, 2007.

4. Rejection under 35 U.S.C. § 102(a)

Claims 6, 7, 14, and 15 are rejected under 35 U.S.C. § 102(a) as allegedly anticipated by Bayramoğlu *et al.*, *Chem. Eng. Sci.* 57: 2323-34 (July 2002) ("Bayramoğlu"). Applicants traverse the rejection as it applies to the amended claims.

Bayramoğlu teaches, "Procion Brown MX-5BR was ***covalently attached*** onto IPNs membrane as a metal chelating reagent." Bayramoğlu, Abstract; *see also* page 2324, heading 2.3, "Procion Brown MX 5BR attachment onto IPNs membrane." In the present claims, the compound of Formula I is not covalently attached to the protein-binding membrane. The cited art thus does not teach each and every element of the claimed invention, and the rejection accordingly should be withdrawn.

5. Rejection under 35 U.S.C. § 103(a)

Claims 1-17 are rejected under 35 U.S.C. § 103 as allegedly obvious over U.S. Patent No. 6,174,729 ("Alam") in view of Hopwood *et al.*, *Histochem. J.* 5: 391-403 (1973) ("Hopwood") and Miyagi, *Seibutsu Butsuri Kagaku* 19: 129-37 (1975) (Abstract) ("Miyagi"). Applicants respectfully traverse the rejection.

Introductory Remarks

Staining procedures known in the art for proteins often involve reagent profiles that are undesirable for reasons including: low sensitivity; lengthy incubation time; reagents that denature or otherwise degrade the analyte proteins, precluding further characterization such as immunodetection; and incompatibility with certain protein-binding membrane compositions. There is thus a need in the art to provide a staining procedure that: (a) is as rapid as ponceau S or amido black 10b staining, i.e., requiring less than ten minutes; (b) employs a dye that is easily removed from the protein-binding membrane in destaining, leaving levels of background stain that do not interfere with protein detection; (c) has a detection limit similar to that achieved by colloidal gold, i.e., capable of allowing detection of proteins down to the nanogram range; (d) employs reagents and conditions that allow staining and destaining without denaturation of the analyte proteins, thus preserving the characteristic antigenic activity, enzymatic activity, etc.; (e) is reversible under conditions that do not denature or otherwise degrade the protein analyte and

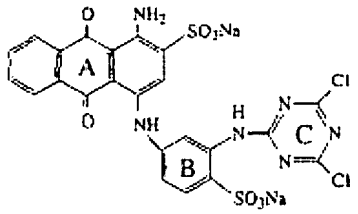
thus preserve the protein's characteristic antigenic activity, enzymatic activity, etc.; and (f) is broadly applicable to different protein substrates and to different protein-binding membranes. *See, e.g.*, Specification, page 3, lines 3-23.

The presently claimed invention addresses these needs by providing *inter alia* a staining method that is rapid and has a detection limit in the nanogram range. The method employs a dye reagent that is rapidly removed from most protein-binding membranes, leaving levels of background stain that do not interfere with protein detection. The method employs reagents and conditions for staining, destaining, and reversal of the staining procedure that do not denature or otherwise degrade protein analytes. Rather, the method preserves any characteristic antigenic or enzymatic activity possessed by the protein. The method is broadly applicable to different proteins and to different compositions of protein-binding membranes. *See, e.g.*, Specification, page 7, lines 18-27.

The Cited Art

Alam teaches visualizing and quantifying a protein on a protein-binding membrane using Coomassie stain. Alam discloses detection of protein bound to a membrane by staining at col. 7, lines 44-58. Alam discloses suitable kits at col. 10, line 57, through col. 11, line 6. The Office acknowledges that Alam does not teach at least the use of a staining reagent according to Formula I, one embodiment of which is Reactive Brown 10.

Miyagi compares the ability of various to stain membrane-bound proteins. The tested dyes are Coomassie Brilliant Blue G-250 ("title dye (I)"), Amido Black 10B, Ponceau 3R, Coomassie Brilliant Blue R-250 (II), and Procion Blue M-RS. Procion Blue M-RS is a dichlorotriazine dye. The cited art does not disclose the structure of "Procion Blue M-RS"; the precise structure of Procion Blue M-RS is unclear from the record. It stands to reason, though, that the structure of Procion Blue M-RS is different from that in Formula I, given the differences in absorbed wavelengths of light between the structures (i.e., blue versus brown). This expectation is reinforced by the structural dissimilarity between Formula I and Procion Blue MX-R (which may or may not be the same compound as Procion Blue M-RS):



Procion blue MX-R

Alderton *et al.*, *Eur. J. Biochem.* 233: 880-85 (1995), at Figure 1 (referencing Hanggi *et al.*, *Anal. Biochem.* 149: 91-104 (1985)).

Hopwood teaches "fixative properties of a number of mono- and dichlorotriazine dyestuffs," where the dyestuffs are used to cross-link proteins in thin slices of rat liver and kidney. Hopwood, Abstract, page 393, ¶¶ 2-3. Hopwood states: "The aim of the present work was to investigate the potential of chlorotriazines as fixatives for tissues." Hopwood, page 400, ¶3. Hopwood teaches the use of the following chlorotriazines:

Colour	*Symbol*
Brilliant Blue	H-3R
Brilliant Blue	M-R
Brilliant Blue	M-3G
Brilliant Orange	M-2R
Brilliant Red	M-2B
Brilliant Red	H-3B
Brilliant Red	M-5B
Brilliant Yellow	H-5G
Brilliant Yellow	M-4G
Brilliant Yellow	M-4R
Brilliant Yellow	M-GR
Brilliant Yellow	M-6G
Grey	M-G
Green	M-2B
Olive Green	M-3G
Red Brown	M-4R
Scarlet	M-G
Red	M-G

Hopwood, page 392. Procion Brown MX-5BR or Reactive Brown 10 is not listed among these Procion dyes.

The Rejection and Response

The Office alleges that it would have been obvious to use Reactive Brown 10 in the method of Alam, because Alam teaches the suitability of any stain. The Office further alleges that the artisan would have known from Miyagi that dichlorotriazine dyes are suitable for staining proteins bound to membranes. The Office finally alleges that "Hopwood teaches that Procyon dyes *such as Reactive Brown 10* are suitable for staining and visualizing proteins in tissue." Office Action, page 5 (emphasis added). The Office alleges that the artisan would have combined the references, based on the allegedly known suitability of dichlorotriazine dyes, including Reactive Brown 10, as suitable alternatives to Coomassie Blue for protein staining. Office Action, page 5 (citing *KSR Int'l Co. v. Teleflex Inc.*, 82 U.SP.Q.2d 1385, 1397 (U.S. 2007)).

The Supreme Court recently has held that a combination of references that is merely obvious to try suffices to establish prima facie obviousness, where there are a finite number of predictable solutions to a problem in the art (*KSR*, 82 U.SP.Q.2d at 1397):

> When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try might show that it was obvious under §103.

KSR addressed the obviousness of an invention directed to gas pedals and sensors. In the context of the generally more unpredictable chemical arts, the Federal Circuit recently has held: "[The] test for prima facie obviousness for chemical compounds is consistent with the legal principles enunciated in *KSR*." *Takeda Chem. Indus. Ltd. v. Alphapharm Pty. Ltd.*, 83 U.S.P.Q.2d 1169, 1174 (Fed. Cir. 2007). The Federal Circuit stated the appropriate test en banc: "structural similarity between claimed and prior art subject matter, proved by combining references or otherwise, where the prior art gives reason or motivation to make the claimed compositions, creates a prima facie case of obviousness." *In re Dillon*, 919 F.2d 688, 16 U.S.P.Q.2d 1897 (Fed. Cir. 1990). That is, in addition to structural similarity between the compounds, a prima facie case of obviousness also requires a showing of "adequate support in

the prior art" for the change in structure. *In re Grabiak*, 769 F.2d 729, 731-32 (Fed. Cir. 1985). The prior art must have suggested making the specific molecular modifications necessary to achieve the claimed invention. *Takeda*, 83 U.S.P.Q.2d at 1174 (citing cases).

In the present case, there is no evidence that the cited art would have suggested the use of a chemical according to Formula I, including the specific embodiment of Reactive Brown 10, in the claimed method. Alam does not teach the use of Formula I, including Reactive Brown 10, as the Office admits. Likewise, Miyagi does not teach the use of Formula I , including Reactive Brown 10, as the Office admits. Miyagi instead teaches the use of structurally distinct compounds, such as "Procion Blue M-RS." The evidence on the record provided above shows that one example of a Procion Blue compound, Procion Blue MX-R, has a different structure from Formula I. Finally, there is no evidence that Hopwood teaches the use of Formula I, including Reactive Brown 10, contrary to the Office's allegation. Notably, the Office's rationale for combining the references is explicitly based in part on the teaching by Hopwood of the use of Procyon dyes, *such as Reactive Brown 10.* Instead, Hopwood provides a list of tested Procion compounds that excludes Reactive Brown 10, or even Pricion dyes that adsorb brown light.

The Office has not provided sufficient evidence to establish a prima facie case of obviousness. The cited art provides no evidence that the compound of Formula I is so structurally similar to Coomassie Blue or the other dyes used by Alam that it could be used in the method of Alam. The cited art provides no evidence whether Formula I is so closely structurally related to Procyon Blue M-RS that is could be used in the method of Miyagi. And the cited art provides no evidence that Formula I is so structurally related to the various Procion dyes used by Hopwood that it could be used in the fixing method of Hopwood. The Office further has failed to show "adequate support in the prior art" to suggest a change in structure from the disclosed compounds to the recited compound of Formula I. *See Grabiak*, 769 F.2d at 731-32. In short, there is no evidence of record at all that the prior art would have suggested making the specific molecular modifications necessary to achieve the claimed invention. *See Takeda*, 83 U.S.P.Q.2d at 1174. Since the Office has not made a prima facie case, Applicants are not required to produce rebuttal evidence detailing the various advantageous properties of the

presently claimed invention. *See, e.g.*, Specification, page 7, lines 18-27. Accordingly, Applicants respectfully request the Office to withdraw the rejection.

CONCLUSION

Should the Examiner have any questions or comments regarding Applicants' response, he is asked to contact Applicants' undersigned representative. Please direct all correspondence to the below-listed address.

In the event that the Office believes that there are fees outstanding in the above-referenced matter and for purposes of maintaining pendency of the application, the Office is authorized to charge the outstanding fees to Deposit Account No. 50-0573. The Office is likewise authorized to credit any overpayment to the same Deposit Account Number.

Respectfully submitted,

CHRISTOPHER R. YONAN, *et al.*

DANIEL A. MONACO
Registration No. 30,480
DRINKER BIDDLE & REATH LLP
One Logan Square
18th and Cherry Streets
Philadelphia, PA 19103-6996
(215) 988-3312 - Phone
(215) 988-2757 - Fax
Attorney for the Applicants